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Evaluation of the Detection of Human Papillomavirus Genotypes in Cervical Specimens by Hybrid Capture as Screening for Precancerous Lesions in HIV-Positive Women

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Given the frequency and persistence of human papillomavirus (HPV) infection and associated cytological alterations in HIV-1-positive women, the incidence of uterine cervix neoplasm is likely to increase along with patient survival. More appropriate screening programs, which, in addition to Pap smears (PS), also include tests to detect and type HPV, are needed for the early identification of precancerous cervical lesions. This prospective study involved 168 HIV-positive (group A) and 100 HIV-negative women (group B). Cervicovaginal samples were collected for a PS and HPV DNA search. The detected virus was typed as high–intermediate oncogenic risk HPV (HR-HPV) and low-risk HPV (LR-HPV) using hybrid capture (HC) (Murex-Digene) and in-house PCR tests. The HC-detected prevalence of HPV was 111/168 (66%:HR 75.6%) in group A and 15/100 (15%:HR 42.9%) in group B ($P < 0.0001$). Polymerase chain reaction (PCR) was positive in 91% and 48%, respectively. No significant difference was observed between drug addicts and heterosexual HIV-1-positive women ($P = 0.09$). HPV was detected in 94% of the 57 HIV-positive women with cytological alterations. HR-HPV was found in 41/49 women with low-grade and 7/8 with high-grade squamous intraepithelial lesions (LSIL and HSIL, respectively). In women with a negative PS, HPV was detected in 57/111 cases (HR 63%) of group A and in 13/98 of group B (6 cases of HR). Of the 54 group A women who underwent biopsy, histology revealed that 41 had LSIL (18 with negative PS, 19 with LSIL, and 4 with HSIL; HR-HPV in 73% and LR-HPV in 17%), nine had HSIL (5 LSIL and 4 HSIL on cytology; HR-HPV in 89% and LR-HPV in 11%), and four were negative (all cytology negative; 3 HR-HPV and 1 LR-HPV). HR-HPV was more frequent as immunodepression worsened. These results show that cytological evaluation alone underestimated histological alterations in 23/50 women (42.6%), whereas the combination of Pap smear and HPV detection reduced this underestimate to 5%. ***J. Med. Virol. 56:133–137, 1998.***

KEY WORDS: human papillomavirus (HPV); human immunodeficiency virus (HIV); precancerous cervical lesions; Pap smear

INTRODUCTION

Cervical neoplasia is about five times more likely to occur in women with HIV-1 infection than in uninfected women [Mandelblatt et al., 1992]. Among Italian female AIDS patients, 1.8% had invasive cervical carcinoma (ICC) as their AIDS-defining condition [Serraino et al., 1996]. Although no data are available on the notification of ICC cases after AIDS definition, it is confirmed that the frequency of cervical carcinoma is higher than in the general population [Schafer et al., 1991; Wright et al., 1994; Sopracordevole et al., 1996]. This emphasizes the need for large-scale preventive programs based on the cervical screening of HIV-infected women. As suggested by the Center for Disease Control, a Pap smear should be carried out at least once a year [Center for Disease Control and Prevention, 1990], but many studies show that the incidence of false-negative findings following a single Pap smear

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in the presence of histologically confirmed cervical precancerous lesions or ICC can be as high as 50%, particularly in HIV-infected women [Syrjanen et al., 1990]. It has been demonstrated that the etiology of ICC is multifactorial, but human papillomavirus (HPV) infection acts as a primary cause [Franco, 1993].

We report the data relating to 168 Italian HIV-infected women studied by a Pap smear and HPV genome detection for the prevalence of HPV infection and its association with precancerous lesions and cancer. An age-matched control group of 100 seronegative women was enrolled in the same geographical area with the aim of investigating the prevalence of HPV types and studying the correlation between Pap smears, the detection of HPV genotypes, and the presence of precancerous lesions.

MATERIALS AND METHODS

Study Population and Specimen Collection

Between March 1995 and January 1997, 168 HIV-seropositive women were recruited in a patient care program at the Department of Infectious Diseases of San Raffaele Hospital, University of Milan. All women are of north Italian origin. Standardized interviews addressed the risk factors for HIV infection. The disease was classified according to the 1993 Center for Disease Control criteria [Center for Disease Control and Prevention, 1992] and the women were divided into three groups on the basis of their CD4 cell counts. One hundred consecutive age-matched HIV-seronegative heterosexuals women of the same area of residence attending the same hospital's clinic for primary cervical cancer screening were selected as a control group. At enrollment, Pap smears and cervicovaginal swabs for the detection of HPV genomic sequences were collected.

Cytology and Colposcopy

The Pap smears were classified according to the Bethesda classification system [Kurman et al., 1994], and all of the women with low- or high-grade squamous intraepithelial lesions (LSIL or HSIL, respectively) were referred for colposcopy. After a preliminary data analysis, colposcopy (with biopsy if necessary) was advised at the first visit.

HPV DNA Detection

Viral genomic sequences were detected by means of a nonamplified hybridization test, the Hybrid Capture (HC) System (Digene Corp., Beltville, MD). In this assay, specimens containing the target DNA are hybridized with two sets of specific HPV RNA probe cocktails: one containing low-risk HPV (LR-HPV) probes (HPV 6, 11, 42–44) and the other containing intermediate-to-high-risk HPV (HR-HPV) probes (HPV 16, 18, 31, 33, 35, 45, 51, 52, 56). A chemoluminescent detection system is used to detect the reaction.

Polymerase chain reaction (PCR) was performed with the aim of increasing the sensitivity of the hybridization test (i.e., for discordant samples with abnormal cytology). We used a set of degenerate consensus primers (MY09–MY11) to amplify a 450 bp region of the L1 gene (encoding for the major capsid protein), which is conserved in HPV types involved in human pathology. The amplified product is then run on a 2% agarose gel to verify the presence of a band of the expected size, then hybridized with oligomeric probes [Bauer and Manos, 1993] or digested with a panel of restriction enzymes (restriction fragment length polymorphism) for viral typing.

Statistical Analysis

Differences in HPV infection were evaluated using the Chi-square test.

RESULTS

Subject Characteristics

The mean age of the 168 HIV-seropositive patients and the 100 seronegative controls was respectively 33.6 (range: 21–62) and 35 years (range: 25–64). The risk factors for HIV infection among the seropositive women were sexual intercourse with HIV-seropositive partners in 54.2% (91/168), and drug abuse in 44% (74/168). Two women were infected by blood transfusion and one declared no risk factor. Thirty-four (20.2%) of the HIV-seropositive women had a CD4 cell count of more than 500/μL, 66 (39.3%) of 200–500/μL, and 68 (40.5%) of less than 200/μL

Cytological Abnormalities, HPV Detection, and Immunosuppression

Figure 1 shows the correlation between the Pap smear results and HPV genome detection by HC in the HIV-1-positive women, subdivided by their CD4 cell counts (upper right, bottom left, and bottom right panels), in comparison with the same parameters obtained in the control group (upper left panel). HPV was detected in 15 of the 100 HIV-seronegative controls (HR-HPV types in 7 and LR-HPV types in 8 cases), and cytology showed two cases of LSIL that were both positive for HR-HPV types. HPV was detected by HC in 111 of the 168 HIV-positive women; the difference between the HIV- positive and -negative women was statistically significant ($P < 0.001$). The PCR-detected prevalence of HPV was 91% in group A and 48% in group B ($P < 0.001$).

The Pap smear was positive in 57 (34%) HIV-seropositive women, 48 (84.2%) of whom had HR-HPV, six (10.5%) LR-HPV, and three no HC-detected virus (but were all three PCR-positive: two with HR-HPV and the third with an untyped virus). Of these women, 17.5% had a CD4 count of more than 500/μL, 33% of 200–500/μL, and 49% of less than 200/μL.

The Pap smear was negative in 111 of the HIV-seropositive women (66%; 24 with CD4 counts of more than 500/μL, 47 with CD4 counts of 200–500/μL, and 40 with CD4 counts of less than 200/μL). Among these women, 57 (51%) were HC-positive for HPV genomic sequences: HR-HPV types were found in 32/57 (56%).

The prevalence of HPV infection in these subpopulations based on HIV risk factors was 73% in drug ad-

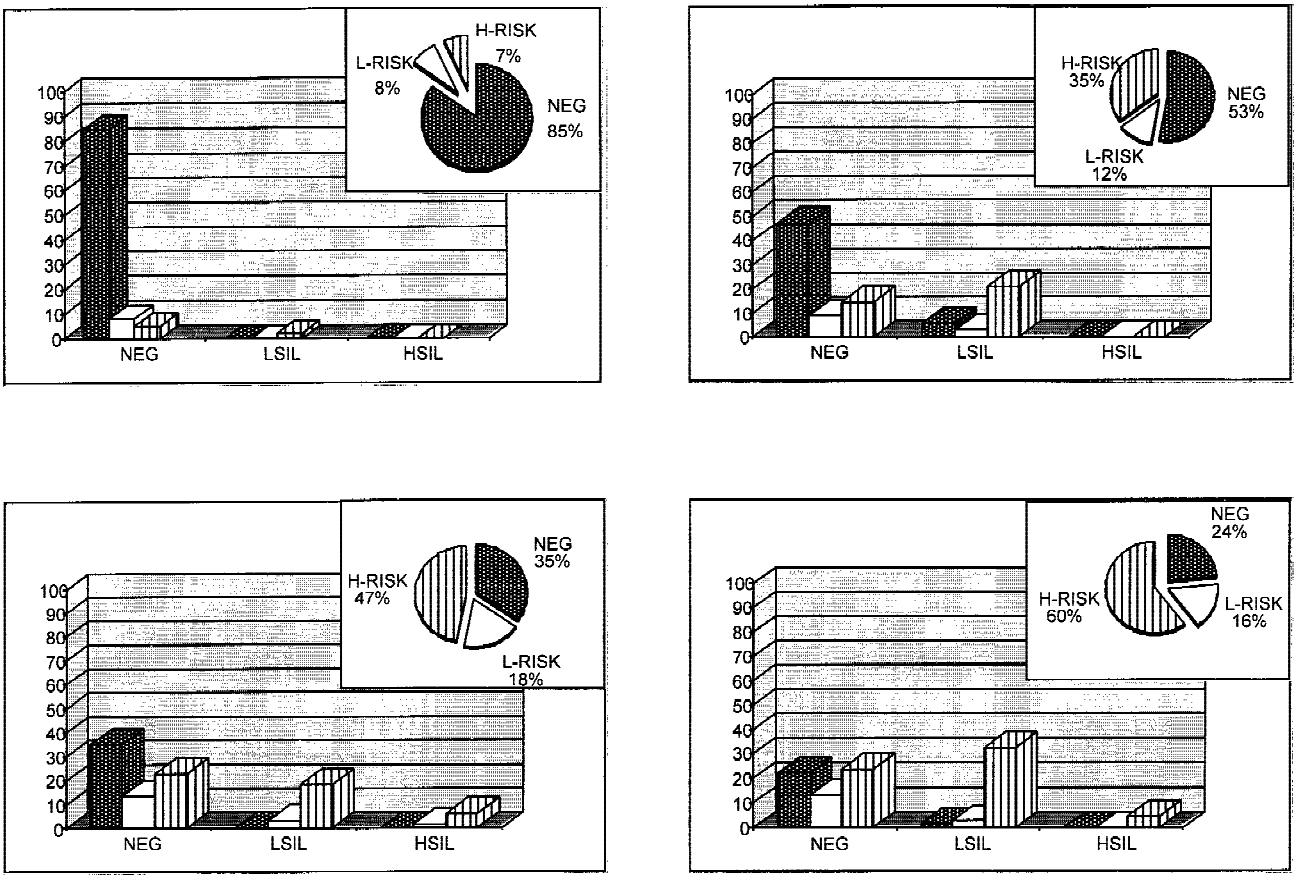

Fig. 1. Correlations between Pap smear results, high-and low-risk HPV detection, and CD4 cell counts. HIV-negative women (**upper left**), HIV-positive women with CD4+ cells > 500/μL (**upper right**), 200–500/μL (**bottom left**), and < 200/μL (**bottom right**).

dicts (77.7% HR-HPV and 22.3% LR-HPV) and 60.4% in heterosexual women (74.5% HR-HPV and 25.5% LR-HPV). As expected, HPV infection was more frequent in drug addicts due to their life style, but the difference between drug addicts and heterosexual women was not statistically significant ($P = 0.09$). LSIL was detected in 16% (15/91) of the heterosexual women and in 23% (17/74) of the drug addicts, and HSIL in 10% (9/91) of the heterosexual women and in 22% (16/74) of the drug addicts.

Correlation Between Cytological and Histological Abnormalities

Fifty-five of the HIV-seropositive women have so far undergone colposcopy: 33 had shown cytological alterations at Pap smear (25 with LSIL and 8 with HSIL), with only one being negative at first-level HPV detection; 22 had shown no Pap test cytological abnormalities (19 of whom were HPV-DNA-positive). One woman underwent colposcopy but not biopsy because of the normal macroscopic examination result: she had an LSIL Pap smear result and was HR-HPV-positive. The remaining HIV-positive women with altered Pap smear results refused to undergo colposcopy or were lost to follow-up.

Histology was positive for LSIL in 41 of the 54 examined biopsies (18 from cases with a negative Pap smear, 19 with LSIL, and 4 with HSIL), and for HSIL in nine (5 with LSIL and 4 with HSIL cytology); no lesions were detected in four cases (all with negative cytology). Of the nine women with HSIL at histology, one had a CD4 cell count of more than 500/μL, five of 200–500/μL, and three of less than 200/μL. Of the 41 women with LSIL at histology, five (12%) had a CD4 cell count of more than 500/μL, 20 (48.7%) of 200–500/μL, and 16 (39%) of less than 200/μL. The four women with a negative histological report were HPV-DNA-positive, one with low- and three with high-risk viruses. Of the nine women with HSIL at histology, eight had HR-HPV and one LR-HPV when tested by HC. LSIL was detected in 60% of the heterosexual women and in 38% of the drug addicts; HSIL in 28% of the heterosexual women and in 55% of the drug addicts.

DISCUSSION

The data confirm the higher prevalence of HPV infection in HIV-positive than in HIV-negative subjects coming from the same area of Italy, a finding that has already been reported by many investigators in relation to HIV-positive women from other countries [Ho et al., 1994; Twedell et al., 1994; Vernon et al., 1995]. The prevalence of viral infection, as well as the importance

of cytological alterations, clearly increases with the severity of immunodepression. The distribution of oncogenic types was similar in the two groups of women and these data reflect the local epidemiology. Furthermore, as many authors have suggested, HIV infection does not increase the susceptibility to oncogenic HPV infections, but the presence of immunosuppression means that the rate of detection is connected with the persistence and/or reactivation of latent infections [Ho et al., 1994; Twedell et al., 1994]. This is confirmed by the results of the present study, which shows that the prevalence of HPV infection in the HIV-positive heterosexual women (subjects who have the same risk factors for HIV-infection as the controls) is similar to that found in the drug addicts. Furthermore, despite the high prevalence of PCR-detected HPV infection in the control population (48%), these women presented a smaller number of cytological alterations (2%) and an extremely limited tendency to develop high-grade SIL.

It is worth noting that, among the cytologically negative women, the prevalence of HPV infection was higher in those who were HIV-seropositive than in those who were seronegative, thus underlining the persistence of HPV infection in these women. Moreover, most of these cases (63%) had an HC-detected HR-HPV infection.

The women with cytologically detected HSIL had HR-HPV more frequently than those with LSIL or negative subjects, thus supporting the correlation between HR-HPV infection and aggressive lesions. The most intriguing observation of this study is the analysis of the virological and histological data relating to the cytologically negative HIV-positive women.

Eighteen women with a normal Pap smear who underwent colposcopy (10 with HR-HPV, 5 with LR-HPV, and 3 with no HPV at HC) were positive for LSIL, thus suggesting the evolutive tendency of HPV infection in HIV-positive women. Five histological cases of HSIL were detected in patients with LSIL Pap smear results, always in the presence of HPV infection (all with HR-HPV). This finding shows that cytological evaluation alone underestimates histological alterations in 42.6% of cases, whereas the combination of Pap smear and HPV detection reduces this underestimate to 5%.

Other investigators showed that the incidence of false-negative findings following a single Pap smear in HIV-positive women with histologically confirmed cervical precancerous lesions can be high. For instance, in the study of Twedell et al. [1994], the calculated false-negative rate for Pap smear was 23% (3 of 13 patients), whereas in the study of Wright et al. [1994], cytology failed to detect cervical intraepithelial neoplasia in 19% of HIV-positive women (15 of 80 patients, 12 had low-grade and 3 had high-grade cervical intraepithelial neoplasia).

These data suggest that the screening of cervical lesions in our population should be integrated with viral study. Another ongoing Italian study of the risk factors for HPV infection suggests that female intravenous drug users (IDUs) have a threefold higher risk of infection than those who are not IDUs [Serraino et al., 1997]. In our prospective study, there was no statistically significant difference in HPV prevalence between the drug addicts and women infected as a result of heterosexual intercourse with HIV-positive partners. The findings suggest that the increased frequency of HPV infection among IDUs with AIDS in Italy may reflect increased HIV testing among women with this risk factor rather than a truly higher prevalence than in non-IDUs. The small number of histological samples does not allow any statistical evaluation of the data to be made; the greater frequency of HSIL in the IDUs (if confirmed in a larger population of subjects and/or by the continuation of the longitudinal study) may be due to other important cofactors for the carcinogenesis of HPV, which are certainly more frequent in IDUs than in heterosexuals, or even to a lower rate of screening for cervical dysplasia among IDUs, as has also been suggested by Simon et al. [1997].

The need for more appropriate screening programs should be emphasized, which would include tests designed to detect HPV and to recognize the presence and persistence of high-risk-type viruses. Our results confirm the fact that a Pap smear alone cannot exclude clinical, subclinical, or latent HPV infections which, in these women, tend to evolve more rapidly (as is demonstrated by the progressively increasing number of low- and high-grade squamous intraepithelial lesions as immunodepression becomes more severe). An approach of this kind would make it easier to ensure the appropriate follow-up and treatment of cervical dysplasia with the aim of reducing the onset of cervical cancer in HIV-positive women.

REFERENCES

Bauer HM, Manos MM (1993): PCR detection of genital human papillomavirus. In Persing DH, Smith TF, Tenover FC, White TJ (eds): "Diagnostic Molecular Microbiology: Principles and Applications." Rochester, MN: Mayo Foundation, pp 407–419.

Center for Disease Control and Prevention (1990): Risk for cervical disease in HIV-infected women in New York City. MMWR 39:846–850.

Center for Disease Control and Prevention (1992): 1993 revised classification system for HIV infection and expanded surveillance of definition for AIDS among adolescents and adults. MMWR 41(RR-17):1–19.

Franco EL, et al. (1993): Human papillomavirus and the natural history of cervical cancer. Infections in Medicine January:57–64.

Ho GYF, Burk RD, Fleming I, Klein RS (1994): Risk of genital human papillomavirus infection in women with human immunodeficiency virus induced immunosuppression. International Journal of Cancer 56:788–792.

Kurman RJ, Henson DE, Herbst AL, Noller KL (1994): Interim guidelines for management of abnormal cervical cytology. JAMA 271: 1866–1869.

Mandelblatt JS, Fahs M, Garibaldi K, Senie RT, Petersen HB (1992): Association between HIV infection and cervical neoplasia: Implications for clinical care of women at risk for both conditions. AIDS 6:173–178.

Schafer A, Friedmann W, Mielke M, Schwartlander B, Koch MK (1991): The increased frequency of cervical dysplasia-neoplasia in women infected with the human immunodeficiency virus is related to the degree of immunosuppression. American Journal of Obstetrics & Gynecology 164:593–599.

Serraino D, Napoli PA, Zaccarelli M, Alliegro MB, Pezzotti P, Rezza G

(1996): High frequency of invasive cervical cancer among female injecting drug users with AIDS in Italy. AIDS 10:1041–1055.

Serraino D, Pezzotti P, Rezza G (1997): HIV exposure category and invasive cervical carcinoma in Italy. AIDS 11:821–822.

Simon PA, Bruce RC, Bunch G (1997): Completeness of reporting of AIDS associated with invasive cervical carcinoma. AIDS 11:820–821.

Sopracordevole F, Campagnutta E, Parin A, Vaccher E, Volpe R, Scarabelli C (1996): Squamous intraepithelial cervical lesions in Human Immunodeficiency Virus seropositive women. Journal of Reproductive Medicine 41:586–590.

Syrjanen S, Saastamoinen J, Chang F., JH, Syrjanen K (1990): Colposcopy, punch biopsy, in situ DNA hybridization, and the polymerase chain reaction in searching for genital human papillomavirus (HPV) infections in women with normal Pap smears. Journal of Medical Virology 31:259–266.

Twedell G, Heller P, Cunnane M, Multhaupt H, Roth K (1994): The correlation between HIV seropositivity, cervical dysplasia, and HPV subtypes 6/11, 16/18, 31/33/35. Gynecologic Oncology 52:161–164.

Vernon SD, Holmes KK, Reeves WC (1995): Human papillomavirus infection and associated disease in persons infected with human immunodeficiency virus. Clinical Infectious Diseases 21:121– 124.

Wright TC, Ellerbrock TV, Chiasson MA, Van Devanter N, Sun X-W, the New York Cervical Disease Study (1994): Cervical intraepithelial neoplasia in women infected with human immunodeficiency virus: Prevalence, risk factors and validity of Papanicolaou smears. Obstetrics & Gynecology 84:591–597.